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Arabidopsis growth and defense are modulated by bacterial quorum sensing molecules

Sebastian T. Schenk, Elke Stein, Karl-Heinz Kogel and Adam Schikora*

Institute of Phytopathology and Applied Zoology; Centre for BioSystems; Land Use and Nutrition; Justus Liebig University Giessen; Giessen, Germany

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N-acyl-homoserine lactones (AHLs) play an important role in the communication within the rhizosphere; they serve as a chemical base for interactions within and between different species of Gram-negative bacteria. Not only bacteria, also plants perceive and react to AHLs with diverse responses. Here we describe a negative correlation between the length of AHLs' lipid chains and the observed growth promotion in *Arabidopsis thaliana*. Moreover, we speculate on a positive correlation between the reinforcement of defense mechanisms and the length of the lipid moieties. Observation presented here may be of great importance for understanding of the complex interplay between plants and their environment, as well as for agronomic applications.

N-acyl-homoserine Lactones Influence Arabidopsis Growth

Diverse reactions can be triggered by AHLs present in the plant's surrounding.[1-8] C6-HSL promotes the growth of *Arabidopsis thaliana* and causes a global transcriptional reprogramming.[5] The transcriptome analysis indicated that many cell wall and cell growth related genes are differentially expressed upon treatment with this short-chain AHL. An altered ratio between the free IAA and cytokinin in treated plant might indicate a shift in the hormonal balance, reflecting the effect of C6-HSL on plant's growth. In contrast, C10-HSL and C12-HSL modulate root development and root hair formation, respectively.[3] However, the relation between altered auxin concentration and AHL was not confirmed in this study, leaving an open question on the exact mechanism of AHL's action. Mathesius et al.[2] showed that upon treatment with different AHLs, one third of the differentially accumulated proteins are specific to the respective AHL, indicating that plants react in a diversified manner to different AHL molecules. Here, we compare the effects of five AHLs with different length of their lipid moieties. The lipid chains of tested molecules range from 6 to 14 carbons. Five-day-old Arabidopsis seedlings were transferred to medium supplied with either 6 μM AHL or acetone (solvent control). Fresh weight of roots and shoots was measured after 11 d. As reported before,[4,5] we observed an increase in shoot and root biomasses upon treatment with C6-HSL (**Fig. 1A**). Moreover, the increase in shoot's biomass caused by AHL was gradually smaller in plants treated with AHLs having longer lipid moieties. This growth-promoting effect was lost when oxo-C12-HSL or oxo-C14-HSL were added to the medium (**Fig. 1A**). These observations were statistically verified using Student's t-test and resulted in a negative correlation between the length of lipid chain and shoot biomass ($y = -0.37x$ and $r^2 = 0.88$). Regarding root's biomass, the situation was even more apparent; only C6-HSL was able to significantly increase the accumulation of Arabidopsis root's biomass (**Fig. 1A**), while treatment with other AHLs provoked no changes. On the other hand, the effect of AHLs on roots resembled the effect on shoot biomasses. Roots' length decreased with increased length of the lipid chain mirroring the negative correlation described above ($y = -1.9x$ and $r^2 = 0.73$) (**Fig. 1B and C**). To enlarge our analysis, we tested also different concentration of AHLs. Already four days after the transfer into AHL-containing media, plants growing on media supplied with 3 μM, 6 μM and 12 μM C6-HSL or oxo-C8-HSL, showed a tendency toward longer roots (**Fig. 1D**). This tendency was not observed in plants treated with oxo-C10-HSL, oxo-C12-HSL (**Fig. 1D**) or oxo-C14-HSL.[4]

AHL Treatment Reinforces Resistance

Recently, we published results suggesting that oxo-C14-HSL, and to lesser extend OH-C14-HSL, induces resistance in Arabidopsis and barley plants toward biotrophic and hemibiotrophic pathogens.[4] Likewise, oxo-C12-HSL also has resistance-inducing potential, though weaker than C14-HSL derivatives (**Fig. 2**). In these experiments sterile grown Arabidopsis plants were treated with AHLs for 3 d and subsequently spray-inoculated with *Pseudomonas syringae* pv *tomato* DC3000. Colony forming units were counted 48 h after inoculation. In contrast to the growth promoting effect, the comparison between oxo-C12-HSL and C14-HSL derivatives, clearly indicates that molecules with longer lipid chains induce stronger resistance (**Fig. 2**). Those

*Correspondence to: Adam Schikora; Email: Adam.Schikora@agrar.uni-giessen.de
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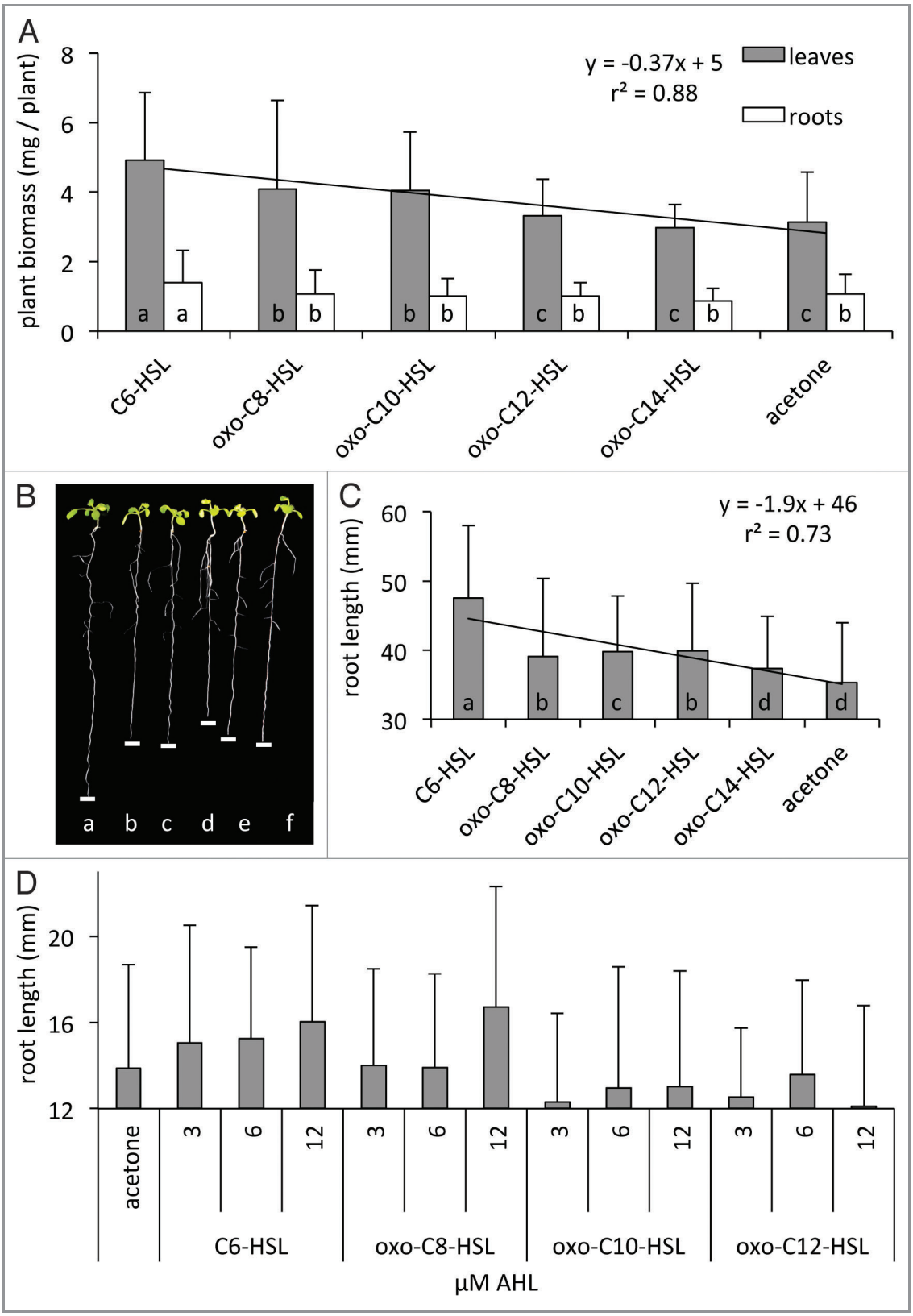

Figure 1. For figure legend, see page 180.

Figure 1 (See previous page). Impact of *N*-acyl-homoserine lactones on plant growth. (A) Increase in biomass of plants treated for 11 d with AHLs differing in the lipid chain lengths. Plants were germinated on a standard one-half MS medium (MS/2)[9] for five days and then transferred for 11 additional days on MS/2 medium supplemented with 6 μM AHL or acetone (as solvent control). Plants were grown in short day conditions at 21°C. Roots and shoots were harvested separately. Small letters indicate the statistical difference calculated with Student's t-test, $p < 0.05$, $n = 45$. (B) Plants grown for 11 d on MS/2 medium supplied with 6 μM of: a) C6-HSL, b) oxo-C8-HSl, c) oxo-C10-HSL, d) oxo-C12-HSL, e) oxo-C14-HSL, or f) acetone. (C) Roots' growth on MS/2 medium supplied with different AHLs. Plants were germinated on a standard MS/2 medium for five days and then transferred for 11 additional days on one-half MS medium supplemented with 6 μM AHL. Root tip position was marked after transfer and used to measure the primary root's growth during the following 11 d. Small letters indicate the statistical difference calculated with Student's t-test, $p < 0.05$, $n = 45$. (D) Root growth on MS/2 medium supplemented with different concentrations of AHLs during the first four days after transfer from the standard MS/2 medium.

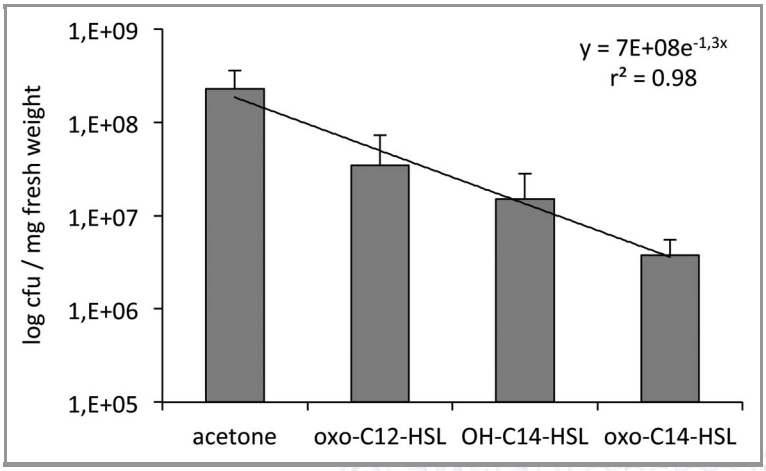

Figure 2. Long-chain AHLs reinforce the resistance to *Pst* bacteria. Arabidopsis plants grown in sterile, hydroponics culture for five weeks were treated with 6 μM of different AHLs for three days and afterwards spray-inoculated with *Pseudomonas syringae* pv *tomato* DC3000 (*Pst*). Colony forming units (cfu) numbers were assessed 48 h post inoculation.

Table 1. Overview on the impact of different AHLs on plant development and physiology. Short-chain AHLs have strong growth-promoting effects. Even though, only root was exposed to AHLs, both shoots and roots show an increase in biomass. In the contrary, long-chain AHLs seem to have no impact on plant development. However, they have strong resistance-inducing capabilities. The AHL-induced resistance is efficient only against biotrophic and hemibiotrophic pathogens[4]

AHL	C6	(oxo)-C8	(oxo)-C10	(oxo)-C12	(oxo)-C14
resistance	-	-	-	↑	↑↑↑
primary root elongation	↑↑↑	↑	↓	-	-
root hair induction	-	-	↑↑	↑	-
secondary root induction	-	-	↑↑	-	-

*Include data from literature: Ortiz-Castro R et al. (2008) Plant Cell Environ 31: 1497–1509; Schikora A et al. (2011) Plant Physiology 157(3): 1407–18; von Rad U et al. (2008) Planta 229: 73–85.

findings are complementary to those published by von Rad et al.,[5] the authors show no resistance induction after treatment with the short-chain C6-HSL. A test for resistance induced with intermediate AHLs, such as C8-HSL or C10-HSL derivatives, should clarify whether only long-chain AHLs induce resistance and determine the minimal chain's length, necessary for this activity (**Table 1**). Many lines of evidence point to a modulated activity of mitogen-activated protein kinases (MAPKs) as the base for AHL-induced resistance.[4] Nevertheless, future experiments are necessary to completely uncover the mechanisms by which AHLs modulate growth and resistance.

Homoserine Lactones Induce Systemic Reaction

A very interesting question is whether the transport of AHLs is a prerequisite for growth promotion. The transport of AHLs within plants has been an issue in several reports. Götz et al.[1] demonstrated the move of C6-HSL and C8-HSL from root to shoot in barley, whereas C10-HSL was not transported. Similarly, C6-HSL, but not the C10-HSL, was translocated from root to shoot in Arabidopsis plants.[5] Recently we showed that C6-HSL but not oxo-C14-HSL, is systemically transported in Arabidopsis from root to shoot.[4] However, the correlation between ability to translocation and growth promotion is still very elusive. The systemically induced resistance is yet another aspect of AHLs' influence on plants. Though we were unable to detect oxo-C14-HSL in shoot after root treatment, oxo-C14-HSL induces resistance against biotrophic leaf pathogens, consistent with the concept of systemic disease resistance. The discovery of the AHL-induced signal(s) will be an important step in the understanding of the mechanism by which *N*-acyl-homoserine lactones influence host plants.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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